DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

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MEMORANDUM

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SUBJECT: Statistical Analysis of the Tumors Observed in Male F1 Rats (Second Parental Generation, P2) at Week 19 in the Argus (1999) Two-Generation Reproduction Study of Ammonium Perchlorate

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This memo addresses your request for statistical analysis of the tumors observed in male F1 rats at 19 weeks of age in the 1999 Argus Laboratories study entitled *Oral (drinking water) two-generation (one litter per generation) reproduction study of ammonium perchlorate in rats. Horsham, PA: Argus Research Laboratories, Inc.* [protocol no. 1416-001].

The tumors were reported by the Pathology Working Group (PWG) convened in 2000 (Experimental Pathology Laboratories, 2000; Wolf, 2000). These data were not available as final for analysis at the 1999 external peer review (U.S. EPA, 1998; Research Triangle Institute, 1999). Wolf (2000) noted that two animals from the high dose group (30 mg/kg-day) in the F1 generation (second parental generation, P2) in the study had adenomas and one of these animals had two adenomas for a total of three. These animals were dosed from conception to 19 weeks of age (adult male F1 rats). The tumors were considered to be treatment related (Wolf, 2000). Compared to the background incidence of thyroid follicular cell adenomas in male F344 rats after 2 years on study at 38/3419 from 67 NTP studies or 1.1% incidence at the 2-year end sacrifice date, this study showed an incidence of 2/30 or 6.7% at 19 weeks.

The purpose of this memo is to provide a more rigorous statistical analysis of the significance that these tumors represent.

Bayesian Statistical Approach:

In order to properly interprete the results from a given toxicological study, it is often necessary to consider the data in light of additional information (outside of the study), such as the variability and average level of response for positive and negative controls in past studies that are similar to the current study. It is also necessary to account for confounding effects that an exposure may have on variables that are associated with the outcome of interest. For example, it is important to adjust for animal survival to avoid bias in analyses of animal tumorigenicity (McKnight and Crowley, 1984) and reproductive toxicity (Dunson and Perreault, 2001). Typically, expert knowledge and information from related studies are accounted for only informally in the interpretation of a statistically significant or non-significant result. However, there are clear advantages to formally incorporating such extra information into the statistical analysis, since it can be very difficult to interprete statistical significance when some aspect of the data is inconsistent with outside information (e.g., the control response is higher or lower than typically seen in related studies). In addition, the formal incorporation of outside information can improve sensitivity and limit bias in assessing toxicological effects. The advantages of including historical control data, in particular, has been well documented in the toxicological and statistical literature (Dunson and Dinse, 2001; Haseman, Huff, and Boorman, 1984; Ibrahim, Ryan and Chen, 1998; Tarone, 1982).

Although frequentist (i.e., non-Bayesian) hypothesis tests can sometimes incorporate historical control data (see, for example, Tarone, 1982), outside information can be incorporated more naturally and flexibly within a Bayesian analysis. In Bayesian analyses, the unknown parameters in a statistical model are assigned prior probability distributions quantifying uncertainty prior to observing data from a current study. For example, based on experience with an assay system, a toxicologist may be 95% certain that the average level of response among vehical control animals is between bounds A and B with C being the most likely value. This information can be formally incorporated into a Bayesian analysis through a prior distribution, for a parameter measuring expected control response, which is centered on C and assigns 95% probability to values between A and B. Alternatively, the prior distribution can be estimated using data or summary statistics for control animals in historical studies if such information is available (Ibrahim, Ryan and Chen, 1998; Dunson and Dinse, 2001). For parameters about which little is known, noninformative or vague prior distributions, that assign equal prior probability to a wide range of plausible values, can be chosen.

Bayesian inferences about toxicological effects can be based on the posterior distribution for the parameters in the statistical model. The posterior distribution, which quantifies the current state of knowledge about the unknown quantities in the statistical model, is

obtained by updating the prior distribution with the information in the data from the current study using Bayes theorem (refer to Gelman et al., 1995 for an overview). One can use the posterior distribution as a basis for conclusions about effects of interest by using posterior means, 95\% credible intervals and posterior probabilities as Bayesian alternatives to the maximum likelihood estimates, 95\% confidence intervals and p-values used in frequentist analyses. For example, as an alternative to a p-value, one could calculate the posterior probability of an increase in the proportion of animals with an adverse response in a treated group relative to the control. Bayesian approaches have been developed for a wide variety of toxicological applications, including risk assessment (e.g., Hill, 1996; Hasselblad and Jarabek, 1996), toxicokinetic modeling (e.g., Bernillon and Bois, 2000), and analysis of skin papilloma data (Dunson et al., 2000).

Argus (1999) Two-Generation Study:

We used a Bayesian approach to assess the effect of ammonium perchlorate in drinking water on thyroid follicular cell adenoma incidence in male Sprague-Dawley rats from a two-generation study (Argus, 1999). The Argus (1999) study dosed male Crl:CD®BR VAF/Plus® (Sprague-Dawley) rats (30 per dose group) with ammonium perchlorate in drinking water at 0, 0.3, 3.0 and 30 mg/kg-day. Statistically significant increases in hypertrophy/hyperplasia were reported for the male F_1 generation at the 3.0 and 30.0 dosages (Argus, 1999).

In the Argus (1999) study, 2 out of 30 male F_1 rats (6.7%) developed 3 thyroid follicular cell adenomas by 19 weeks of age after treatment with 30 mg/kg/day ammonium perchlorate, with one of the rats getting two of these tumors. Although statistically significant decreases in colloid were reported at both the 3.0 and 30.0 mg/kg/day dose levels (Argus, 1999), none of the rats in the other groups (0, 0.3, 3.0 mg/kg/day) developed thyroid follicular cell adenomas (0/30, 0/30, 0/30, respectively).

Without incorporating historical data on spontaneous neoplasms in Sprague-Dawley rats, the difference between 0/30 in the vehicle control and 2/30 in the 30 mg/kg/day group is non-significant by standard tests (e.g., Fisher's exact). However, the reported historical control incidence of thyroid follicular adenomas for male Sprague-Dawley rats in 2 year studies is approximately 3-4% (Chandra et al., 1992; McMartin et al., 1992), suggesting that these tumors should be extremely rare among 19 week old animals in the absence of a treatment effect. Without formally incorporating this historical information into the statistical analysis through a prior distribution, it is very difficult to assess the weight of evidence in favor of a treatment-related increase in thyroid follicular adenoma incidence.

Choosing Prior Distributions Based on Historical Controls:

The proportion of control male Sprague-Dawley rats developing thyroid follicular cell adenomas in 2 year carcinogenicity studies has been reported in the literature. Chandra et

al. (1992) reported a rate of 48/1340 (3.6%), and McMartin et al. (1992) reported a rate of 23/583 (3.9%). In order to incorporate this historical control data into our analysis of the effect of ammonium perchlorate on thyroid incidence at 19 weeks of age, we follow a Bayesian approach. The historical data can be summarized using a Beta(71,1852) prior distribution for the probability of a male Sprague-Dawley rat developing a thyroid follicular cell adenoma (in the absence of treatment with a test agent) by the time of natural death or sacrifice at 2 years. The Beta prior is the standard choice for a prior distribution on a probability (c.f., Dunson and Tindall, 2000 and Gelman et al., 1996 for further discussion of the Beta prior). The values 71 and 1923 are simply the numbers of control male Sprague-Dawley that do and that do not develop thyroid follicular cell adenomas, respectively, from the Chandra et al. (1992) and McMartin et al. (1992) articles.

To account for the fact that the Argus (1999) study recorded thyroid incidence at19 weeks and not at the time of natural death or sacrifice at 2 years, we chose a prior distribution for the ratio of the probability of thyroid follicular cell adenomas at 19 weeks to the lifetime probability in a 2 year study. Portier, Hedges and Hoel (1986) suggest that the probability of a control male Fischer 344/N rat developing a thyroid follicular cell adenoma increases approximately in proportion to age$^{4.78}$. Based on this estimate and on the average survival time for male Fischer 344/N rats in the NTP historical control database (95.2 weeks), our prior expectation for the ratio is $(19/95.2)^{4.78}$ = 5e-04. Allowing for a high degree of uncertainty in this prior expectation due to uncertainty in the Portier, Hedges and Hoel (1986) estimate and in extrapolation from Fischer 344/N rats to Sprague-Dawley rats, we choose a Beta(0.11, 2.6) for the ratio. This prior has median 5e-04 and 95% interval (0,0.379).

Results:

Using the prior described in the previous subsection and "updating" the prior with control data from the Argus study (i.e., 0 tumors out of 30 control male rats), we estimate that a control rat has a 0.15% chance of developing a thyroid follicular cell adenoma by 19 weeks. In addition, had perchlorate had no effect on the incidence of thyroid follicular cell adenomas, the probability of observing two or more rats with these tumors out of 30 would be approximately 0.005. Thus, the data strongly support the hypothesis that ammonium perchlorate in the drinking water at 30mg/kg/day causes an increase in the incidence of thyroid follicular cell adenomas.

Summary:

Incorporating historical control data in a Bayesian analysis, we find a significant increase in thyroid follicular cell adenoma incidence at 19 weeks in male Sprague-Dawley rats relative to controls. There was no evidence of an increase at low dose levels.

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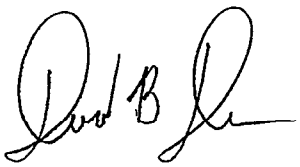

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